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Cytogenetic prognostication of acute myeloid leukemia from a tertiary care hospital in Chennai

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ABSTRACT

Introduction: Acute myelogenous leukemia (AML) comes under the class of haematological malignant disorders. Karyotypic aberrations at diagnosis are crucial for the assessment of the response to medication and the recognition of an initial recurrence of the disease. *Materials and Methods*: Thirty-five de novo subjects of myelogenous leukemia were enrolled in the study. Examination of AML was based on morphology on bone marrow (BM) aspirates; cytochemistry and chromosomal analysis were executed by unstimulated cultures using the regular cytogenetic technique. *Results:* There were 21 females and 14 males with an age group ≥ of 16 years. Cytogenetic investigation of these subjects showed normal karyotype in 7 (20%) patients and aberrant karyotype in 28 (80%) patients. Cytogenetic data in AML was grouped into three types: favourable risk, intermediate-risk, and unfavourable risk. Cases in the standard-risk group react well to the chemotherapy while cases with intermediate and unfavourable karyotype had relapsed. *Conclusion*: We endorsed that cytogenetics will be achieved frequently in all subjects of AML. During the investigation, molecular markers should be combined with cytogenetic studies for risk stratification to enhance the therapeutic scenarios.

Keywords: Acute myelogenous leukemia, Bone Marrow, Cytochemistry, Haematological disorder, Karyotyping.

1. INTRODUCTION

AML is extremely prevalent in adults and reproduction of clonal immature blast cells in the blood and bone marrow (Arber et al., 2016). AML is a disorder of hematopoietic stem and progenitor cells characterized by complex aberrant karyotypes due to genetic mutations (Stölzel et al., 2016) and epigenetic dysregulation leading to somatic alterations in coding regions of genes or enhancer elements (Wouters & Delwel, 2016). AML is otherwise known as acute myeloid leukemia, acute granulocytic leukemia, acute myeloblastic leukemia or acute nonlymphocytic leukemia (Sharma et al., 2012). WHO has classified a variety of AML risk stratification factors like good, intermediate, and poor-risk prognosis. In general, a good prognosis is

associated with long-term five-year survival (LTFY) of up to 65%, the intermediate prognosis is combined with LTFY of about 25%, and poor-risk disease is associated with LTFY of less than 10% (Chauhan, 2018). Advanced research in cytogenetics helps us to know the morphology and clinical heterogeneity of AML. On the basis of karyotype, AML has been subdivided into favorable (16% of cases) consisting of inv(16), t(8;21), t(15;17), and t(16;16); intermediate (20% of cases) includes deformities which does not come either in favorable or unfavorable karyotypes, and unfavorable (13% of cases) includes add (5q), add (7q), del (5q), del (7q), inv (3), t (3;3), t (6;11), t (9;22), t (10;11), 17p deformities, monosomies 5 or 7, monosomy 17 etc (Grimwade et al., 2010).

Molecular inherited examination of intermittent translocations and inversions of chromosomes lead to clone genes adjoining to chromosome breakpoint and also to define their protein products associated in the leukemogenesis conversion (Chilton et al., 2014). Based on cytogenetics examination, it is a highly clonal disorder, contains greater than a hundred intermittent chromosomal deformities. Karyotypic assessment of myelogenous leukemia is a convenient tool determining the proliferation of AML, exclusively when there is specific treatment therapy and at the evaluation of a hematopoietic stem cell transplant (Naeem, 2005). Cytogenetic test may help in stratifying the patients to a more tailored treatment therapy. This could provide the updated knowledge for practising clinical hemato-oncologist.

This retrospective study was performed to evaluate the recurrence of AML-associated karyotypes, cytogenetic deformities cases with myelogenous leukemia and to compare the precise chromosomal deformities with clinical and lab specification in these subjects.

2. MATERIALS & METHODS

Study Design

This study was designed as a retrospective study and conducted as per the institution ethical principles after obtaining proper consent. A newly recognised thirty-five cases of AML with age ≥ 16 years were enrolled in this study.

Screening of Chromosomal Aberrations

Recruitments of Samples

Bone marrow Aspirates were collected from thirty-five newly diagnosed AML cases. This study was done between April 2017 and April 2020. The study was authorized by the Institutional ethical committee, (IEC No. 002/ SBMH/ IHEC/2016/ 206) and carried out at the Tertiary Care Hospital in Chennai.

Morphology Identification

Morphology of AML was identified by using Bone Marrow Aspirates (based on French-American-British (FAB) Classification) (Singh et al., 2017) with the help of cytochemical stains like Sudan Black B (SBB), Myeloperoxidase (MPO) and Nonspecific esterase (NSE) (Gupta et al., 2019).

Sample preparation, harvesting & Karyotype Analysis

The cytogenetic examination was performed by the G-banding method. Bone marrow cells Karyotype were achieved by using specific cytogenetic techniques. Marrow aspiration was collected in sodium heparin vacutainer and cultured in RPMI1640 cell culture medium with 40% fetal calf serum. The cultures were incubated at 37°C overnight. Mitotic stimulants were not used. Colcemid (0.01 mg/mL) was added to the cultures for 15 minutes and followed by hypotonic treatment using 0.075 M KCl for 10 minutes at 37°C. The fixation was done with a 3:1 ratio of absolute methanol: glacial acetic acid solution with 10-minutes. Chromosome slide preparation was using Giemsa staining (G-Banding), (Falini et al., 2005) airs dry the slide for metaphase analysis and investigation of chromosomal deformities should require at least 20 metaphaseswere analyzed for chromosomal abnormalities (Loghavi et al., 2014).

3. RESULTS

In this study, we examined 35 de novo cases of AML and there were 21 females and 14 males (ratio 1.5:1) with age ≥ 16 years. The clinical characteristics showed 24(68.5%) patients had a fever, 30(85.7%) patients had pallor, 23 (65.7%) patients had bleeding manifestation like epistaxis and gingivitis. Hepatomegaly was present in 13(37.1%) cases, Splenomegaly was present in 14(40%) cases, and Lymphadenopathy was present in 8(22.8%) patients. Clinical characteristics of male, female and percentage of AML depicted in figure 1.

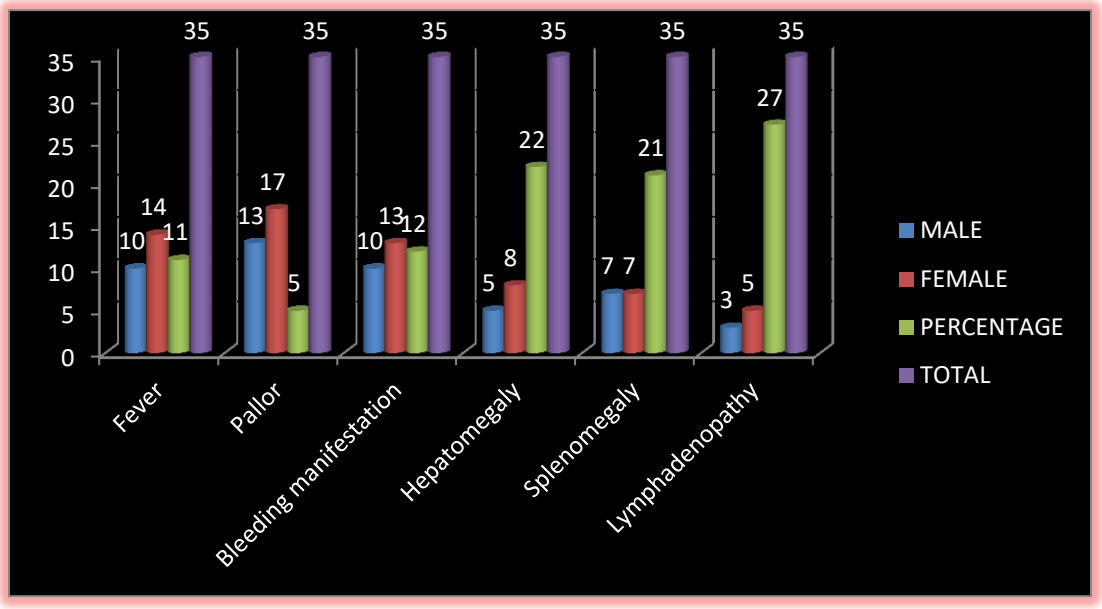

Figure 1 Clinical Description of Acute Myeloid Leukemia (AML)

During the examination, Total leukocyte count ranged from 1.3-170 × 10^6/µl (mean 25.2× 10^6/µl), haemoglobin 2.3 -10 gm% (mean 6.7 gm%), platelet count 10–150 × 10^6 /µl (mean 65.1 × 10^6 /µl) and Bone marrow blast count differ from 20% to 90% (mean 58%). Investigation of both myeloperoxidase (MPO) and Sudan Black B (SBB) shows positive for all the blast cells. In-case of nonspecific esterase (NSE) shows only M4 and M5 blasts. Biochemical, Haematological, and cytogenetic description of AML exhibited in table 1.

Table 1 Biochemical, Haematological and Cytogenetic description of AML

S.NO	Age/Sex	TLC*10^6/µl	Hb g%	Platelet*10^6/µl	Blast %	Diagnosis	Cytogenetics	Cytochemistry
1	42/M	170	10.1	150	80	AML	46,XY/47,XY+21	MPO,SBB
2	53/F	5.6	5.4	60	62	AML - M4	46,XX,inv(16)(p13;q22)	MPO,SBB,NSE+
3	17/F	3.9	3.6	20	33	AML	46, XX, t (8;21)(q22;q22)	MPO,SBB
4	18/M	1.9	8.5	85	20	AML	AML, XY	MPO,SBB
5	35/F	14	6.8	49	60	AML	46,XX,t(9:22)(q34;q11.2)	MPO,SBB
6	70/F	3.4	9	12	67	APML	46,XX,t(15:17)(q24;q21)	MPO,SBB
7	42/M	4.5	7.5	34	89	AML	AML,XY	MPO,SBB
8	55/F	8.6	10.6	10	70	AML	46, XX, t (8;21)(q22;q22)	MPO,SBB
9	71/F	8.8	2.3	100	72	AML	46,XX,t(9:22)(q34;q11.2)	MPO,SBB
10	36/F	160	8	78	33	AML	46, XX, t (8;21)(q22;q22)	MPO,SBB
11	44/F	5.5	4	150	20	AML	46,XX,inv(3)(q21;q26.2)	MPO,SBB
12	23/F	4.6	4.7	90	80	AML	AML,XX	MPO,SBB
13	27/M	9.3	6.9	49	45	AML M5	46,XY/47,XY+8	MPO,SBB,NSE+
14	56/F	3	3	120	88	AML	46, XX, t (9;11)(p21;q23)	MPO,SBB
15	72/F	2.3	7.8	60	92	AML - M4	46,XX,inv(16)(p13;q22)	MPO,SBB,NSE+
16	45/M	10.5	6.1	85	65	AML	46, XY, del (3)(q21q26)	MPO,SBB
17	37/F	1.33	8.4	60	43	AML	46, XX, t (8;21)(q22;q22)	MPO,SBB
18	28/M	28.3	10.3	28	86	APML	46,XY,t(15:17)(q24;q21)	MPO,SBB
19	60/F	65.4	2.3	67	46	AML	46,XX,t(9:22)(q34;q11.2)	MPO,SBB
20	47/F	13	9	89	50	AML	46, XX	MPO,SBB
21	53/F	8.4	5.4	46	70	AML	46, XX, del	MPO,SBB

							(12)(p11;p13)	
22	18/M	9.2	9.2	55	20	AML	46, XY	MPO,SBB
23	27/M	50.8	8.8	30	57	AML M5	47, XY,+8, del (11)(q23)	MPO,SBB,NSE+
24	62/M	3.9	3.8	100	33	AML	46, XY, t (8;21)(q22;q22)	MPO,SBB
25	31/F	1.9	7.2	120	68	AML	46,XX/47,XX,+21	MPO,SBB
26	40/M	14	5.6	53	90	AML	46, XY, t (8;21)(q22;q22)	MPO,SBB
27	29/M	3.4	10	75	45	AML	46,XY,inv(3)(q21;q26.2)	MPO,SBB
28	19/F	5.5	3.5	40	33	AML	46, XX	MPO,SBB
29	42/M	4.6	8.3	35	80	AML	46, XY, t (8;21)(q22;q22)	MPO,SBB
30	27/F	9.3	2.7	28	45	AML	46, XX, t (9;11)(p21;q23)	MPO,SBB
31	34/M	140	3.6	69	27	AML	46, XY	MPO,SBB
32	63/F	34	8.4	88	44	AML	46,XX,t(9:22)(q34;q11.2)	MPO,SBB
33	40/F	55.7	10.3	90	86	AML	46, XX, t (8;21)(q22;q22)	MPO,SBB
34	21/F	18.4	7.5	30	77	AML	46, XX, t (9;11)(p21;q23)	MPO,SBB
35	29/M	1.6	8.3	25	60	AML	46,XY,t(9:22)(q34;q11.2)	MPO,SBB

Karyotype examination showed Cytogenetically Normal (CN) AML in 7 (20%) patients and an unusual karyotype in 28 (80%) patients. Cytogenetic arrangement in myelogenous leukemia was grouped into three types namely: favourable risk, intermediate-risk, and unfavourable or poor risk. In this study, there were 12 (34%) patients come under the favourable risk group and cytogenetic anomalies include patients with the translocation (t) (8; 21), inversion (inv) (16), and the t (15; 17). There were 7 (20%) patients in the intermediate-risk group and deformitiescontain only in the myelogenous leukemia with trisomy 8, trisomy 21, and t (9:11). The unfavourable or poor-risk group includes 9 (26%) cases with inv (3), t (9:22), and deletion. Figure 2 showed the cytogenetics frequency of AML. Myelogenous leukemic cases come under the standard-risk group react efficient to the chemotherapy while in cases with intermediate and unfavorable karyotype the disease had recurrent and most of the cases had failed to investigate.

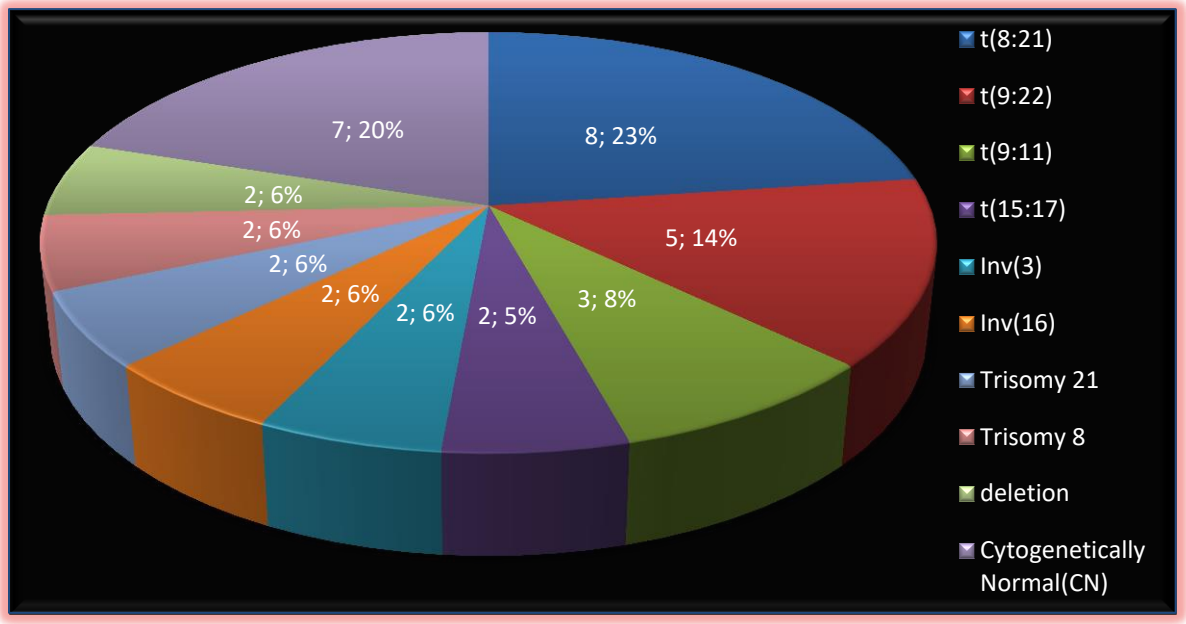

Figure 2 Cytogenetic frequency of Acute Myeloid Leukemia (AML)

4. DISCUSSION

Myelogenous leukemia is a haematological disorder identified based on the clinical, morphological, immunophenotypic and connected structural & numerical deformities (Cheng et al., 2018). The subtyping of myelogenous leukemia is derived from the

morphologic and cytochemical method of the previous French-American-British (FAB) systems that scrutinize the decision of karyotypic interpretation (Meng et al., 2013). Even though morphological investigation of BM aspiration part as an essential requisite for the examination of myelogenous leukemia, it is notable that the presence or absence of precise cytogenetic anomaly and genetic variation is not only effective for deciding prognosis but is also appropriate for therapy guidance (Alrajeh et al., 2017). AML is a malignant blood disorder, rapidly generating neoplasm of immature haematopoietic blast cells. Repetitive chromosomal rearrangements such as t (8; 21) (q22; q22), t (15; 17) (q22; q12), and inv (16) (p13q22) are commonly described as deformities in AML (Gupta et al., 2019). Chromosomal aberration in AML is divided into copy number variation and structural variation. Numerical variation appears due to the fact of chromosome missegregation, during the cell division, which leads to the loss or gain of particular chromosomes (Williams & Amon, 2009). Untreated myelogenous leukemic patients will die within a week, due to the accumulation of a large amount of immature cells in the marrow and blood. Cytogenetics is accepted as one of the great extensive relevant prognostics determinates in AML. Unusual karyotype has been identified in relatively 60% of myelogenous leukemic subjects have favourable cytogenetics that involves t (8; 21), t (15; 17) and inv (16); these cases have 90% complete remission (CR) rate and 65% of five-year survival rate (Grimwade et al., 2010).

The present investigation mainly assesses the recurrence of cytogenetic deformities in cases with myelogenous leukemia and interact precise chromosomal variations with clinical and laboratory specification these subjects. Our findings of clinical parameters ratio like fever (68.5%), pallor (85.7%), bleeding manifestation (65.7%), Hepatomegaly (37.1%), Splenomegaly (40%) and Lymphadenopathy (22.8%) and also we observed the mean values for haematological parameters likes, total leukocyte count (mean 25.2× 10^6/µl), haemoglobin (mean 6.7 gm %), platelet count (mean 65.1 × 10^6 /µl) and Bone marrow blast (mean 58%). There were 12 (34%) patients comes under the favourable risk group, 7 (20%) cases in the intermediate-risk group and the unfavourable risk group includes 9 (26%) cases.

5. CONCLUSION

In conclusion, cytogenetics analysis plays a crucial role in the risk stratification and regimen of AML cases. This correlation study must be accomplished with numerous parameters likes, biochemical and haematological and BM morphology. During the investigation, molecular markers should be combined with cytogenetic studies for risk stratification to enhance the therapeutic scenario. We state hereby that cytogenetics will be achieved frequently in all cases of acute myelogenous leukemia. In the current investigation, a total of 35 cases were examined with ≥ 16 years'age groups. AML with t (8; 21), t (15; 17) and inv (16) forecasted as a good-risk group in comparison to the complex karyotypes. In the case of a good-risk group, an autologous or allogeneic Stem Cell Transplantation (SCT) will be performed for AML recurrent cases after drug therapy. The study shows the significance of diagnostic cytogenetics as a self-reliant prognostic determinant in AML.

Cytogenetics is designed as one of the great relevant prognostic determinants in myelogenous leukemia and the somatic chromosomal variants are played a crucial role in haematological malignancies. Conventional cytogenetics mainly used for routine banded chromosomal analysis to get an overview of the human genome. Currently, molecular cytogenetics approaches mainly for describing the structure and function of a chromosome. The extensive investigation of all cytogenetics deformities requires a unification of techniques such as karyotyping, Fluorescence in Situ Hybridization (FISH) and Copy Number Variation (CNV)-microarrays and these higher resolution techniques to directly map the genome copy number variation.

Recently studies have been reported that not only cytogenetic abnormalities but also genetic deformities are associated to the molecular mechanisms involved in myelogenous leukemia and clinical prognosis. In future, taking into account the cytogenetic, molecular genetics approach will assist us to create a more extensive picture of the underlying pathophysiology of the disease. This will, therefore would lead to the identification of various types of novel variations which will undoubtedly boost the diagnosis, prognosis, monitoring, as well as promoting targeted therapy decisions and which would hopefully reduce health costs, morbidity and mortality in AML patients.

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Authors Contribution

All authors (Suguna Elumalai, Chitralekha Saikumar & Florida Tilton) have made a substantial, direct, and intellectual contribution to the research work, and approved it for publication.

Informed consent

Written & Oral informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this manuscript.

Ethics statement

The study has been approved by the Institutional Human Ethics Committee, Sree Balaji Medical College and Hospital and was carried out in accordance with the standard guidelines (Ethical approval code - IEC No. 002/ SBMH/ IHEC/2016/ 206).

Conflicts of interest

The authors declare that they have no conflict of interest.

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This study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

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